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The effect of activated charcoal and number of species offered on intake of Mediterranean shrubs by sheep and goats

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Abstract

Maquis and garrigues are extensive shrubland vegetation types in the Mediterranean region that provide important habitat for wild and domestic herbivores. Although the majority of these shrubs are nutritious, virtually all contain secondary compounds that reduce their forage value. Understanding how animals cope with ingesting secondary compounds is vital for increasing consumption of shrubs and for enhancing and maintaining biodiversity of these shrublands. Ingesting compounds such as activated charcoal can ameliorate the negative effects of secondary compounds and enable animals to eat more shrubs, but offering a variety of shrubs may have a similar effect on increasing intake. Thus, our objectives were to determine if supplemental charcoal and numbers of shrub species offered influenced intake of shrubs by sheep and goats. We conducted two trials each with 12 sheep and 12 goats (six activated charcoal versus six controls). In Trial 1, we offered six shrubs. In Trial 2, we initially offered three shrubs (Period 1: *Juniperus phoenicea*, *Helichrysum italicum* and *Juniperus oxicedrus*), then two shrubs (Period 2: *J. phoenicea* and *H. italicum*), and finally one shrub (Period 3: *J. phoenicea*). In Trial 1, goats ate more total shrub biomass than did sheep (45.7 ± 1.0 g/kg b.w. versus 27.2 ± 0.7 g/kg body weight (b.w.), respectively), and there was no difference in shrub intake

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($P = 0.39$) between charcoal-treated animals and controls (mean 37.5 ± 0.8 g/kg b.w.). In Trial 2, supplemental charcoal had a positive effect on total shrub intake for both sheep and goats in all three periods (Period 1, $P < 0.001$; Period 2, $P < 0.001$ and Period 3, $P < 0.03$), and goats ate more shrubs than did sheep in all three periods ($P < 0.01$). Regardless of period, both species of animals showed a numerical decrease in total shrub intake, with or without supplemental charcoal, as the number of shrub species on offer decreased. Our findings support the hypothesis that plant biochemical diversity plays an important role in diet selection, thus enabling animals to better meet their nutritional needs and avoid toxicity. In addition, as the number of shrubs in the diet decreased, and supplemental energy was reduced, activated charcoal had a greater impact on shrub intake. Finally, activated charcoal had the same influence on sheep and goats throughout the trials, even though goats always ate more shrubs than did sheep.

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1. Introduction

In the Mediterranean basin shrubs and low-growing trees <5 m high – collectively referred to as “maquis” – are widespread, throughout the region, including the Adriatic littoral of Croatia which covers over 60 million ha (Le Houerou, 1980). In Croatia and elsewhere, these traditional grazing areas are important sources of forage for sheep and goats, particularly during the dry summer (Rogosic, 2000). However, use of Mediterranean shrublands is often limited by secondary compounds such as terpenes, which at too high concentrations can adversely affect forage intake and animal health (Estel et al., 2000; Banner et al., 2000; Villalba et al., 2002a).

Terpenoids are the largest group of plant secondary chemicals (15,000–20,000 currently fully characterized), with a common biosynthetic origin in mevalonate (Langenheim, 1994). Some research suggests that terpenoids deter herbivores by inhibiting cellulolytic activity of rumen microbes (Nagy et al., 1964). However, other research suggests that terpenes are rapidly absorbed from the gut before negatively affecting rumen microbes (Welch and McArthur, 1981; Welch et al., 1983). In the latter case, the physiological effect of terpenes may limit forage intake to a greater extent than simply through anti-microbial activity (Foley and McArthur, 1994; Dziba et al., 2006).

Animals use different strategies to regulate intake of toxins and to cope with ingested toxins (Provenza, 1996; Provenza et al., 1994). Intake of shrubs with high levels of secondary compounds such as terpenes is regulated by both serum and tissue concentrations and excretion rates (Foley and McArthur, 1994; Dziba et al., 2006). If the excretion rate of toxins is slow the potential toxicity and metabolic cost of the detoxification are increased (Foley et al., 1987), and acid base balance may be disrupted (Illius and Jessop, 1997). This increase in organic acid load may initiate negative feedback and deter shrub intake by sheep and goats (Foley et al., 1995), though there is evidence that feedback from the toxin per se may be more important (Dziba et al., 2006).

Secondary compounds in too high concentrations limit how much of any particular food an animal can eat, and there are ways animals can cope with secondary compounds

(reviewed in Villalba and Provenza, 2005). One is to ingest substances that reduce the toxicity of the compounds or that enhance the ability of animals to detoxify the compounds. For instance, polyethylene glycol binds to tannins, reducing their aversive effects, and activated charcoal can increase use of shrubs rich in terpenes by adsorbing terpenoids, thereby reducing their negative effects. In addition, supplementing livestock with energy and protein can facilitate detoxification processes and increase intake of a food high in secondary compounds. Another strategy to reduce the effects of toxins is to eat a variety of plants that differ in their kinds and amounts of secondary compounds.

To date, little research has been done to determine how supplements such as activated charcoal and the number of species offered may interact to influence food intake. Thus, our objectives were to determine if intake of shrubs by sheep and goats increased if they were supplemented with activated charcoal and if the number of shrub species offered influenced feed intake. We determined if the effect of supplemental charcoal was consistent when animals were offered different combinations of shrubs or a single, less palatable and terpene-rich shrub (*Juniperus phoenicea*).

2. Materials and methods

2.1. Study shrubs and animals

Two trials were conducted at a research station 25 km from Split, Croatia, on the Central Adriatic coast. The experiments were conducted during autumn (October–November 2003) and spring (April–May 2004). In Trial 1, we offered six dominant shrubs: *Quercus ilex* L. (Fagaceae), *Erica multiflora* L. (Ericaceae), *Arbutus unedo* L. (Ericaceae), *J. phoenicea* L. (Cupressaceae), *Viburnum tinus* L. (Caprifoliaceae), and *Pistacia lentiscus* L. (Anacardiaceae). Trial 2 was divided into three periods. In Period 1 we offered *J. phoenicea* L. (Cupressaceae), *Helichrysum italicum* (Roth) Guss. (Asteraceae) and *Juniperus oxycedrus* L. (Cupressaceae). In Period 2 we offered *J. phoenicea* L. (Cupressaceae) and *H. italicum* (Roth) Guss. (Asteraceae). In Period 3 we offered only *J. phoenicea* L. (Cupressaceae). *J. phoenicea*, *H. italicum* and *J. oxycedrus* contain terpenes (Salido et al., 2002; Mezzetti et al., 1970), whereas *Arbutus unedo*, *Q. ilex* and *P. lentiscus* are high in tannins (Rogosic et al., 2003, 2006a). Iridoid glycosides and terpenoids in *V. tinus* (Tomassini et al., 1995) also limit intake by sheep and goats. Both *Q. ilex* and *E. multiflora* contain spines, but in neither case did spines appear to deter feeding (Rogosic et al., 2006a).

The experimental sheep ($n = 12$, mean weight 23.08 ± 1.59 kg) were a local breed that is a cross between the Croatian breeds “Pramenka” and “Wunterberg”. The goats ($n = 12$, mean weight 19.83 ± 1.89 kg) were a mixture of domestic goats crossed with Saanen and Alpine breeds. Both species of animals had an approximately equal mix of both sexes. All experimental animals were raised on the same farm on the island of Brac (Central Dalmatia) utilizing the shrubby vegetation of the Mediterranean maquis. The animals generally maintained their body weight during the trials.

All shrubs offered to sheep and goats were hand-harvested each week in the same location. Leaves and 1-year old twigs, 10 cm long, were clipped and placed in bags. Within an hour the plant material was ground to 1 cm length with a chipper, mixed for uniformity,

placed in woven, polyethylene feed sacks, and refrigerated at 4 °C. Every morning before the trial, bags of shrubs to be fed that day were removed from cold storage and offered immediately to animals. Standard nutritional analyses were conducted on samples of plant material during a previous study, and are reported in Rogosic et al. (2006a).

2.2. Conditioning

Animals housed individually (1.5 m × 2 m pens) in covered stalls with wire mesh sides, and fresh water and trace mineral salt were provided free-choice. Prior to the experiments baseline intake of alfalfa pellets was determined for each animal on days 1–5. This allowed us to evaluate if any toxic effects on intake were evident from previous shrub consumption, and to minimize carry-over effects. After baseline was established, all animals underwent a preconditioning period where 20 g of commercial grade activated charcoal mixed with 180 g ground barley was given for 5 days to accustom animals to the supplement and to facilitate initial acceptance of the shrubs and reduce the possibility of forming taste aversions (Banner et al., 2000).

After this preconditioning period, all animals were fed 180 g ground barley mixed with 20 g of activated charcoal from 08:00 to 08:30 and offered all six shrubs simultaneously from 09:00 to 15:00 for 3 days. We monitored shrub intake daily. Then we divided 12 goats and 12 sheep into one treatment group that was given activated charcoal, and one control group/species (controls versus activated charcoal— $n = 6/\text{species}$). We ranked the animals on the basis of their shrub intakes over the 3-day trial and used all odd ranks as one treatment. This procedure balanced initial shrub acceptance across treatment groups and reduced experimental variability (McIntyre, 2005). Further, this modified ranked set approach provides unbiased estimates with equal or greater precision compared to simple random sampling (Dell and Clutter, 1972). Treatment groups were unaltered during the trials.

2.3. Trial 1: influence of activated charcoal on intake of six Mediterranean shrubs offered to sheep and goats

The objective of the first trial was to assess the effects of activated charcoal on intake of six dominant shrubs of the Mediterranean maquis by sheep and goats (Table 1).

Table 1
Shrubs and supplement provided per animal (i.e., sheep or goats) during the study

Trials	Shrubs offered	No supplemental activated charcoal (control group)	Supplemental activated charcoal (treatment group)
Trial 1	6	200 g barley + alfalfa (70% energy diet)	180 g barley + alfalfa, 20 g activated charcoal (alfalfa 70% energy diet)
Trial 2			
Period 1	3	100 g barley	100 g barley, 20 g activated charcoal
Period 2	2	0 g barley	100 g barley, 20 g activated charcoal
Period 3	1	0 g barley	100 g barley, 20 g activated charcoal

At 08:00 h each morning on days 1–10 treatment sheep and goats received 180 g ground barley mixed with 20 g of activated charcoal, whereas control animals were offered 200 g ground barley. Then, all animals were offered 200 g of each shrub simultaneously in separate food boxes from 09:00 to 15:00 h daily for 10 days. Animals and food boxes were checked every 30 min and additional ground material was added as needed. Food refusals were collected and shrub consumption was calculated. At 15:00 h animals were offered varying amounts of alfalfa pellets calculated to provide 70% of their maintenance energy requirements (including barley; INRA, 1989).

2.4. Trial 2: influence of activated charcoal on intake of varying numbers of Mediterranean shrubs offered to sheep and goats

2.4.1. Trial 2, Period 1: three shrubs offered to sheep and goats

The objective of this trial was to determine the effect of activated charcoal on shrub intake when the number of shrubs was reduced incrementally along with supplemental energy and protein (Table 1). During Period 1, three shrubs were fed simultaneously (*J. phoenicea*, *H. italicum* and *J. oxycedrus*). Unlike in Trial 1, we did not offer alfalfa pellets after 15:00, and the amount of barley given daily was reduced from 200 to 100 g, while the amount of activated charcoal remained the same. During the 6-day trial, treatment animals were given a 100 g mixed preload of ground barley with 20 g activated charcoal at 08:00, while control animals received only 100 g preload of ground barley. All animals were then fed 200 g of each shrub from 09:00 to 15:00 h. Additional shrub material was added as necessary every 30 min until 15:00. Each day refused shrubs were collected and biomass consumption was calculated.

2.4.2. Trial 2, Period 2: two shrubs offered to sheep and goats

During period 2, we offered two shrubs (*J. phoeniceae* and *H. italicum*) from 09:00 to 15:00 for 5 days. Refusals were collected and weighed, and intake of shrubs was determined. Supplemented sheep and goats received 100 g ground barley mixed with 20 g activated charcoal, while controls did not receive ground barley or activated charcoal in this period. No animals received alfalfa pellets.

2.4.3. Trial 2, Period 3: one shrub—*J. phoenicea* offered to sheep and goats

During Period 3 only one shrub (*J. phoeniceae* L.) was offered to sheep and goats for 5 days. All procedures of this trial were otherwise the same as in Period 2. Each day refused shrubs were collected and biomass consumption was calculated.

2.5. Statistical analysis

The total daily amount consumed of all shrubs offered in each trial was used as the dependent variable in the analysis. The experimental design for the activated charcoal trials was a completely random design. Animals were a random factor in the mixed model analysis (SAS, 2000). The model included treatment (activated charcoal versus control), species of animal (goats versus sheep), and the species \times treatment interaction; individual animals were nested within treatment and species. The model also used days as a repeated

measure with all other interactions included. All analyses on shrub intake were adjusted for body weight (g/kg b.w.).

3. Results

3.1. Trial 1: influence of activated charcoal on intake of six Mediterranean shrubs offered to sheep and goats

Goats and sheep differed in total shrub consumption ($P = 0.0008$; Fig. 1). Regardless of treatment, goats ate substantially more shrubs than did sheep (45.7 ± 1.0 g/kg b.w. versus 27.2 ± 0.7 g/kg b.w., respectively). Charcoal-treated animals and controls did not differ in total shrub intake ($P = 0.39$; mean 37.5 ± 0.8 g/kg b.w.). There was also a day effect ($P = 0.001$), but no significant interactions. In general, all animals tended to increase intake from the first to the last day of the trial.

Even though sheep and goats differed in total amounts of shrubs eaten, the rank order of the amount eaten for each shrub species (i.e., preference order) was essentially the same for both sheep and goats. The mean amounts eaten of the shrubs across all treatments and animal species were: *E. multiflora* (11.2 g/kg b.w.), *V. tinus* (10.8 g/kg b.w.), *Q. ilex* (8.1 g/kg b.w.), *Arbutus unedo* (5.4 g/kg b.w.), *P. lentiscus* (1.3 g/kg b.w.) and *J. phoeniceae* (0.7 g/kg b.w.).

3.2. Trial 2, Period 1: three shrubs offered to sheep and goats

As in Trial 1, goats again ate more shrubs than did sheep throughout Trial 2 ($P < 0.01$, Fig. 2). Charcoal-treated sheep and goats ate more total shrubs than did control animals

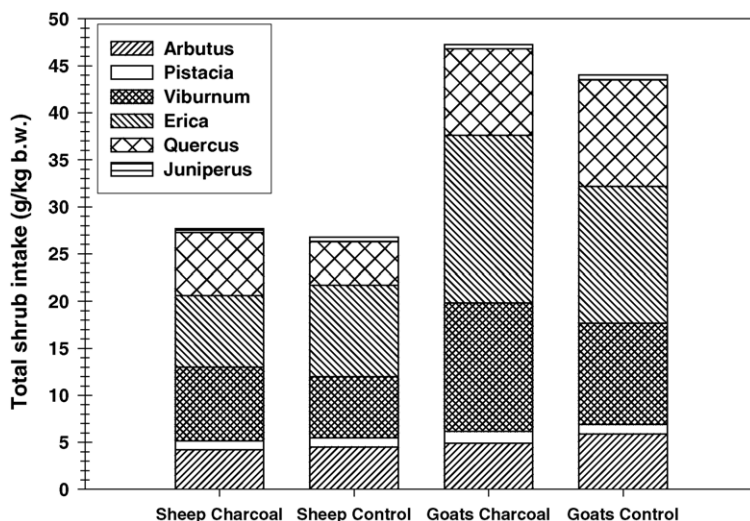


Fig. 1. Intake by sheep and goats offered six shrubs simultaneously and treated with charcoal, and untreated controls. There was no effect of charcoal ($P > 0.05$) and goats' shrub intake was greater ($P < 0.01$) than sheep. Standard errors for total shrub intake are given in the text.

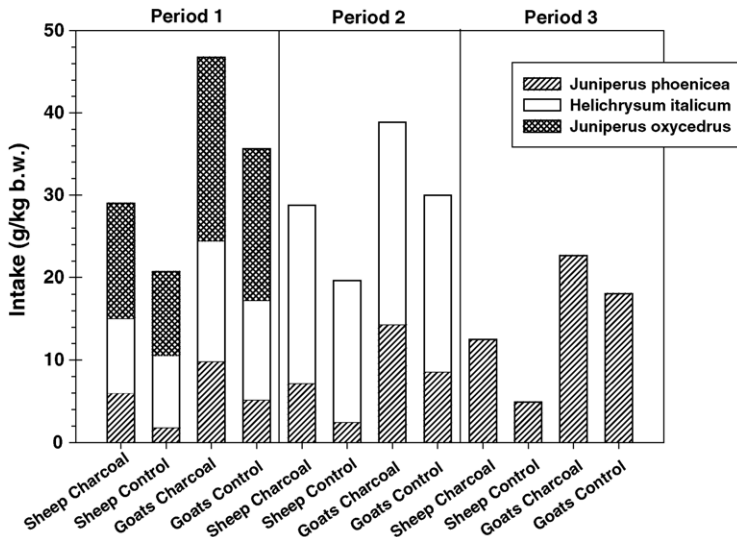


Fig. 2. Intake by goats and sheep supplemented (charcoal) or not (control) with activated charcoal when offered three, two or one species of shrub; $n = 12$ in each trial. In each period, goats' shrub intake was greater ($P < 0.01$) than sheep, and charcoal-treated animals consumed more shrubs ($P < 0.05$) than did controls. Standard errors are given in the text.

($P = 0.002$; 37.9 ± 1.4 g/kg b.w. versus 28.2 ± 1.3 g/kg b.w.). There were no species \times - treatment or treatment \times day interactions ($P > 0.50$), but there was a species \times day interaction as both goats and sheep increased intake of the three shrubs over the period ($P = 0.004$; Fig. 3).

3.3. Trial 2, Period 2: two shrubs offered to sheep and goats

Goats had a higher total intake when offered two shrubs than did sheep ($P = 0.0003$; 34.5 ± 0.9 g/kg b.w. versus 24.3 ± 0.9 g/kg b.w.; Fig. 2). Sheep and goats fed charcoal ate more shrubs than did control animals ($P = 0.001$; 33.9 ± 0.8 g/kg b.w. versus 24.8 ± 1.1 g/kg b.w.). There were no species \times treatment or species \times day interactions ($P > 0.50$), but there was a treatment \times day interaction ($P = 0.03$) as charcoal-treated animals increased intake of the two shrubs over the 5-day period more than the control animals (Fig. 3).

3.4. Trial 2, Period 3: one shrub—*J. phoenicea* offered to sheep and goats

Goats ate more ($P = 0.001$) *J. phoenicea* (20.4 ± 1.09 g/kg b.w.) compared to sheep (8.7 ± 0.70 g/kg b.w.). There was a treatment \times day ($P = 0.03$) interaction as charcoal-treated animals, particularly sheep, increased intake of *J. phoenicea* over time compared to control animals (Fig. 3). There was also a species \times day interaction ($P = 0.02$) as sheep increased intake over the period more than did goats even though sheep ate less *J. phoenicea* biomass compared to goats (Figs. 2 and 3).

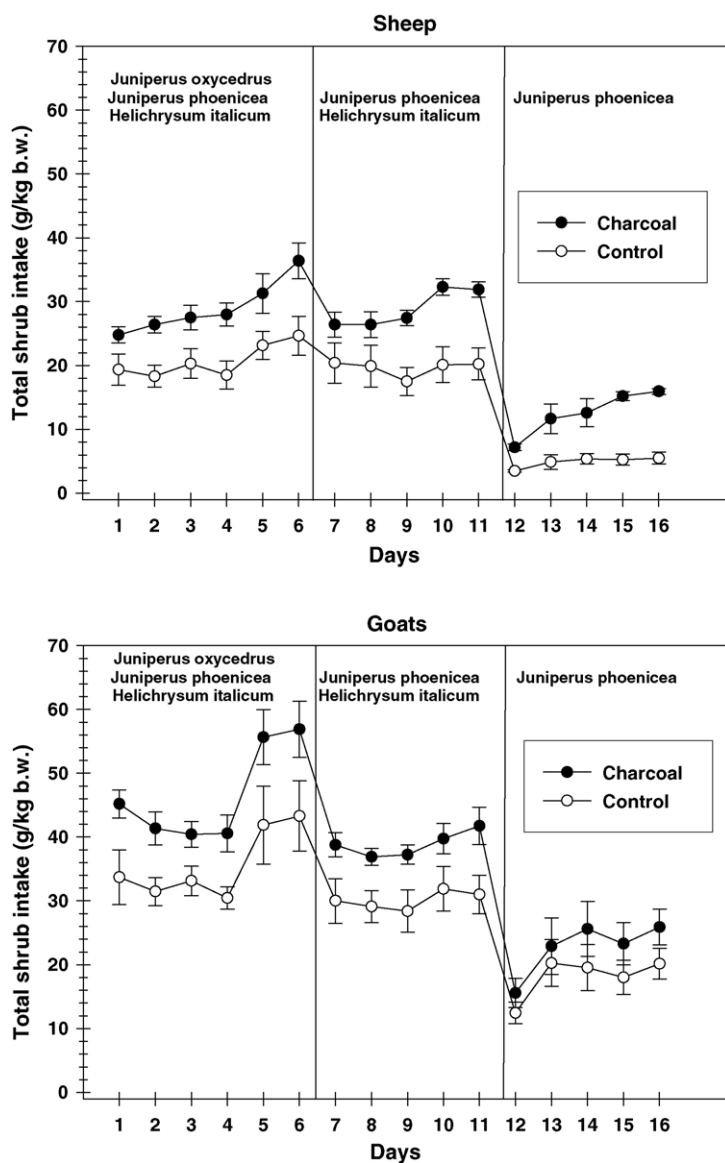


Fig. 3. Average daily intake by goats and sheep supplemented or not with activated charcoal when offered three, two or one species of shrub; $n = 12$ in each trial.

4. Discussion

4.1. Influence of biological/biochemical diversity on shrub selection

The vast majority of Mediterranean shrubs contain secondary compounds (Tisserand and Alibes, 1989; Van Soest, 1994), thus browsing sheep and goats can not avoid ingesting

various secondary compounds that commonly occur in those plants (Bryant et al., 1991). Most secondary compounds are ingested in amounts too low to cause acute symptoms of poisoning and death, and we saw no overt adverse signs of toxicity. Such signs would include digestive disturbance, rumen stasis, depression, anorexia, and in advanced cases neurological effects and ataxia (Burrows and Tyrl, 2001). This study confirmed the common view that mixed diets improve food intake (Freeland and Choquenot, 1990). The kinds and different amount of nutrients and toxins in the Mediterranean shrubs fed to sheep and goats probably influenced animal intakes, which in browsing animals is probably dynamically determined by the physiological state of animal relative to the quality and quantity of nutrients and toxins available (Villalba et al., 2002a). As the number of shrubs decreased from Trial 1 through the three periods in Trial 2, the concentration of terpenes in shrub biomass offered to animals probably increased, so total shrub intake decreased numerically for both sheep and goats. In other words, these results clearly showed that combinations of more shrubs offered to animals promoted greater intake. These results are consistent with the satiety hypothesis (Provenza et al., 2003) that interactions among flavors, nutrients, and toxins (i.e., secondary compounds) lead to enhanced intakes.

Depending on prevailing conditions, sheep and goats browsing on Mediterranean shrubby vegetation may be able to mix their diets to provide sufficient energy and protein while reducing toxin loads. The shrubs offered in these experiments varied in amounts of macronutrients (Rogosic et al., 2006a), and also in kinds and amounts of secondary compounds such as terpenes (high terpenes: *J. phoenicea*, *H. italicum* and *J. oxycedrus*), tannins (high tannins: *Arbutus unedo*, *Q. ilex* and *P. lentiscus*), and iridoid glycosides in *V. tinus*. A mixture of biochemically diverse plant species is likely to enable herbivores to eat a combination of foods, and an animal's preference may range along a continuum from strongly aversive, if nutrients and toxins are not complementary, to strongly positive if nutrients and toxins are complementary (Burritt and Provenza, 2000). Plant secondary metabolites that affect different organs or are detoxified by different pathways are likely to be less toxic when ingested as a dilute mixture than when each toxin is ingested in a larger dose (Foley et al., 1999). Brushtail possums maintain a higher intake of food when allowed to select from two diets containing phenols and terpenes than when given access to the diets individually (Dearing and Cork, 1999). Sheep and goats displayed similar ingestive behavior with the shrubs offered in this study (Fig. 1; Trial 1).

Our results suggest that interaction between macronutrients, activated charcoal, flavor and toxins influenced how sheep and goats mixed their diets and utilized shrubs, and that supplemental macronutrients enhanced intake of food that contain terpenes and tannins, as found in other studies (Banner et al., 2000; Villalba et al., 2002a). Likewise, different concentrations of nutrients in plant species may have different effects on selection of food by sheep and goats depending on the classes and concentration of toxins in the plant community. Relationships among shrubs are likely to vary on a case-by-case basis depending on the biochemical composition.

4.2. Effect of charcoal on Mediterranean terpenes-rich shrubs intake

Activated charcoal adsorbs phytochemicals in shrubs and facilitates their excretion via the feces (Buck and Bratich, 1986). Positively charged surfaces in activated charcoal bind

with the negatively charged molecular surface of many toxins, decreasing their bioavailability (Poage et al., 2000). In Trial 1, activated charcoal did not enhance use when six shrubs were offered simultaneously to sheep and goats. Complementary interactions among nutrients and toxins in the different shrubs species and in the basal diet apparently negated the need for activated charcoal. Animals in this trial were provided more supplemental feed than in the subsequent trial, and this may have positively influenced total shrub intakes.

During Trial 2 (Period 1), sheep and goats supplemented with 100 g barley + 20 g activated charcoal (treatment groups) ate more shrubs than sheep and goats supplemented with 100 g barley (control group) when we offered 3 shrubs. This is similar to other results when charcoal-supplemented lambs ate more sagebrush than lambs supplemented only with barley (Banner et al., 2000).

When three shrubs were offered to sheep and goats, and controls were given energy as well, there was a positive effect from charcoal on intake of three shrubs high in terpenes (*J. phoenicea*, *H. italicum* and *Juniperus oxicedrus*; Trial 2, Period 1). When two shrubs (*J. phoenicea* and *H. italicum*; Trial 2, Period 2) or one shrub (*J. phoenicea*; Trial 2, Period 3) was offered to sheep and goats, activated charcoal and energy together substantially increased intake of shrubs high in terpenes (Figs. 2 and 3). Previous work has shown that lambs that learned to eat foods containing a variety of toxins ate more when they could select two of the foods rather than only one (Villalba et al., 2004).

Further, goats and sheep receiving charcoal and energy in the 3- and 2-shrub period ate much more shrub than did controls, indicating the effectiveness of charcoal and barley in reducing the impacts of terpenes from *J. phoenicea*, *H. italicum* and *J. oxicedrus*. In Periods 2 and 3 the control animals did not receive supplemental feed. Thus, the effects of charcoal and energy in Trial 2, Periods 2 and 3, are confounded because controls had neither supplement. We view the confounding as a minor issue because the relative magnitude of the treatment effects remained similar between all periods during Trial 2. We attribute most of the treatment effect to activated charcoal.

4.3. Comparative responses of sheep and goats

Sheep and goats differed in their intakes of shrubs, as shown in other studies (Rogosic et al., 2006a,b). Differences in anatomy and physiology help to account for dissimilarities in shrub selection. Goats have an incisor arcade that is narrower and more pointed than that of sheep (Gordon and Illius, 1988). Likewise, goats also can achieve higher bite rates than sheep, perhaps due to higher chewing efficiency or willingness to swallow larger particles (Domingue et al., 1991). Sheep and goats also may differ in ruminal metabolism of toxins (Kronberg and Walker, 1993) and biotransformation of absorbed toxins (Villalba et al., 2002b). Likewise, difference in ruminal fermentation and adaptation of rumen microbes to terpenes also may enable goats to more efficiently use terpenes-rich foods (Landau et al., 2000).

Sheep and goats also responded similarly in several respects. In general in Trial 2, both sheep and goats increased intakes of shrub as each period progressed, indicating ongoing adaptation to the shrubs. Even though goats generally ingested higher levels of shrubs than

did sheep in both trials, goats and sheep both responded similarly to supplemental charcoal. Finally, as the number of shrub species offered decreased, intake of shrubs decreased for both goats and sheep. In another similar trial, supplemental polyethylene glycol (PEG) had a greater influence on sheep than on goats when three Mediterranean shrubs were available, and PEG had the most influence on both sheep and goats when only one or two foods were available (Rogosic et al., 2006b).

5. Conclusions

Sheep and goats are primary consumers of the Mediterranean shrubby vegetation, and thereby shape the diversity, structure and dynamics of these extensive ecosystems. Sheep and goats foraging on Mediterranean shrublands have the potential to either increase or decrease species diversity and abundance. In this study, as the number of shrub species in the diet increased, so too did intake. When sheep and goats were offered six shrubs and given a higher plane of nutrition, activated charcoal alone had no effect on shrub intake. Charcoal supplement positively influenced shrub intake when plant species were restricted to three or fewer. Activated charcoal had the same relative influence on sheep and goat intakes throughout the trials, and charcoal had the most influence on both sheep and goat intakes when shrub and energy availability were reduced.

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